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# Evaluation of the Effect of Extracellular Polymeric Substance (EPS) from *Lactobacillus plantarum* (LP) on the Disease Resistance of Rice Field Eel (*Monopterus albus*) Fingerlings

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The experiment aimed to determine the appropriate level of EPS to supplement in the feed *M. albus* to help increase their resistance to diseases caused by the *Edwardsiella tarda* T4 strain. The experiment included 6 treatments, with 3 repetitions, consisting of 2 control treatments: positive and negative control; and 4 treatments with supplements of 4 g/kg, 6 g/kg, 8 g/kg, and 10 g/kg of feed, conducted at the aquaculture facility of An Giang University. The experiment was conducted over a period of 6 weeks using fingerling *M. albus* with an average weight of 3–5 g per fish. Each experimental tank was stocked with a density of 250 fish per tank. The results of monitoring environmental factors such as pH, temperature, and dissolved oxygen were all suitable for the growth of *M. albus*, with values ranging from 25.9 °C to 30.6 °C for temperature; 4.8 to 6.2 mg/L for dissolved oxygen (DO); and pH ranging from 7.58 to 8.03. The results showed that supplementing with an EPS level of 8 g/kg of feed after 4 weeks was the most effective compared to the other treatments ($p<0.05$). The survival rate (SR) reached 96.67% after exposure to the pathogenic bacterium *E. tarda* T4, and the effectiveness of EPS usage relative percentage survival (RPS) was 88.89%. The density of red blood cells and white blood cells reaches the highest levels, respectively 3.03 x $10^6$ cells/mm$^3$ and white blood cells 1,404.63 x $10^3$ cells/mm$^3$; lymphocyte 2,464.35 x $10^3$ cells/mm$^3$ and monocytes 185.84 x $10^3$ cells/mm$^3$. The recorded results indicate that EPS helps stimulate the immune system of the eel and has the potential to prevent disease. Thus, EPS produced from LP bacteria has the ability to resist diseases caused by the *E. tarda* T4 strain on *M. albus*, with the most suitable dosage being 8g/kg of feed for 4 consecutive weeks.
**Keywords:** An Giang University, rice field eel fingerling, E. tarda T4, extracellular polymeric substance (eps), hematological parametres.

## INTRODUCTION

The rice field eel (*Monopterus albus*) is one of the aquaculture species that has garnered significant attention recently due to its delicious meat, stable prices, and high-income potential for local people. Currently, the freshwater eel is becoming one of the most favored export products in foreign markets such as China, Hong Kong, and Japan. However, during the breeding and farming processes, there are many challenges related to diseases and the quality of the fingerling. Among these, the quality of the fingerling and the occurrence of diseases are the primary factors determining the success of the farming season. Many studies have focused on the disease in the eel including according to the survey results by Quyen (2020), eel disease is considered the most significant challenge in the breeding process (66.7%), and 60% of households believe that the survival rate from fry to fingerling is still low with a survival rate of 50-60%. Some research results on eel diseases indicate that freshwater eels are very susceptible to bacterial infections during the fingerling stage. According to Hang (2012) five groups of bacteria were identified in diseased eel samples, including *A. hydrophila*, *Aeromonas* spp., *E. tarda*, *Lactococcus* spp. and *Streptobacillus* spp. Additionally, Oanh and Hien (2012) identified *Aeromonas hydrophila* as the main pathogen causing hemorrhagic disease in freshwater eels. In recent studies its identified that *E. tarda* is a primary pathogen

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which is responsible for hemorrhagic disease, with ulcers on the fingerling freshwater eels (Hang *et al.*, 2024). *E. tarda* is a common pathogen for various aquaculture species and causes considerable economic losses in aquaculture farming (Goh *et al.*, 2023; Adeshina *et al.*, 2024). According to Sherif *et al.* (2020), the bacterium *E. tarda* is a one of main pathogens in fish, that causes high mortality rates and losses in aquaculture. Prebiotics commonly are food supplementations, which are not digestible but stimulate the growth or metabolism of the desirable gut bacteria with skewing to colonization of beneficial bacteria, and they possess the capability to promote organisms' gut equilibrium (Gibson *et al.*, 1995). Prebiotics selectively feed microorganisms and through this has selective stimulation on the host's gut microbiota, and this mechanism has well documented in large animals, poultry, and aquaculture. Supplementation of prebiotics to eel feed shows that they affect growth rate, feed conversion rate, gut microbiota, resistance against pathogens, and enhance immune defense (Gibson *et al.*, 2005). Another study stated that, the use of prebiotics not only leads to better immune responses, and may also have the potential optimizing the efficiency of aquaculture production and output (Ringø *et al.*, 2010). While, extracellular polymeric substances (EPSs) are known to have prebiotic properties with promoting the growth and colonization of beneficial bacteria species in the intestine, EPSs were found to enhance the cellular immune responses of the shrimp, with optimizing total haemocyte count, respiratory bursts, phenol-oxidase activity, and superoxide dismutase (SOD) activity (Thanh *et al.*, 2025). Also, similar report has been published for fish species. Based on published researches on substances which have immunostimulatory-effects in disease occurrence and with increasing survival rates are documented in different animal species such as shrimp and catfish, with using β-glucan, inulin, and synbiotics Thanh *et al.* (2021); Hang and Phuong (2020); Zhou *et al.* (2020). Based on these backgrounds, present experiment aimed to determine the appropriate level of EPS to supplement in the feed *M. albus* to help increase their resistance to diseases caused by the *Edwardsiella tarda* T4 strain.

## MATERIALS AND METHODS

***Materials*:**

- The weighed of the eel is approximately 2 – 5 g/con.
- EPS-LP is produced from *Lactobacillus plantarum* bacteria (Thanh *et al.*, 2024)
- *Edwardsiella tarda* T4 bacteria are stored at -80 °C in the laboratory of An Giang University, Vienam National University, Ho Chi Minh, Vietnam. The bacteria have an $LD_{50}$ of $2{,}1 \times 10^4$ CFU/mL (Hang *et al.*, 2024).
- Tryptone Soy Agar (TSA, Merck) medium.
- Chemicals: Nutrient Broth (NB, Merck) for bacterial growth.
- Some tools and equipment in the laboratory. Experimental tank measuring 60 cm x 60 cm x 40 cm and black mesh substrate.

***The experiment on the effect of EPS on the survival rate, prebiotic utilization efficiency (RPS), and blood cell density of the M. Albus*:** The experiment was completely randomized, consisting of 6 treatments and 3 repetitions. The fingerling eels were placed in tanks for 5 to 7 days to acclimate to the experimental environment, with a stocking density of 250 fingerling eels per tank.

The eels were fed experimental diets with 4 levels of EPS-LP: 4 g/kg, 6 g/kg, 8 g/kg, and 10 g/kg of feed, and were fed with 2 supplementation intervals of 2 weeks and 4 weeks continuously.

Control treatments: Negative: Fingerling eels were not fed feed mixed with EPS-LP and were not exposed to pathogenic bacteria; Positive: Fingerling eels were not fed feed mixed with EPS-LP and were exposed to pathogenic bacteria.

After continuous feeding for 2 weeks and 4 weeks, the eels were subjected to infection with the pathogenic strain *E. tarda* T4 at a dose of $LD_{50}$ $2{,}1 \times 10^4$ CFU/mL. The experiment was monitored for 14 days, recording determining the effectiveness (RPS) of EPS, as well as the red and white blood cell counts in the fingerling eels' blood.

***Water quality care and management*:** The experimental eels were fed twice a day. The feeding amount was 5% - 7% of body weight per day. The experimental eels were given feed from CJ Feed and Care Company, which has a protein content of 40%. Prebiotics were dissolved in water and then evenly sprayed and mixed into the feed before being given to the eels. Leftover feed and waste were collected about one hour after feeding the eels using a siphon, and the water was changed twice a day before feeding the eels again about one hour later. Environmental factors were monitored: Dissolved Oxygen (DO), pH, temperature, Ammonia ($NH3/NH_4^+$), and nitrite ($N\text{-}NO_2^-$) during the experiment (Boyd and Pillai, 1985).

***Criteria*:** All fish were weighted and measured for weights and before and after the experiment. Relative percentage survival (RPS); red blood cell (RBC) and white blood cell (WBC) total were calculated using the equations.

RPS (Relative percentage survival) = [1 - (percent of mortality eel in experimental treatment/ percent of mortality eel in experimental positive control] *100 (Ellis, 1988)

**Total RBC = C × 10 × 5 × 200 (cell/mm$^3$).**

Total WBC (cell/mm$^3$) = (number of WBC in 1.500 cell × R)/number of RBC in 1.500 cell

Density of the type of white blood cell (cells/mm$^3$) = (Number of each type of white blood cell × Average Count)/200

***Statistical analysis*:** Statistical analyses were conducted using the general linear model procedure (GLM) of the Minitab 16.0 software. Means and standard errors were calculated.

One-way ANOVA and Tukey tests were used to compare means between treatment groups.

## RESULTS

***Results of monitoring water environmental factors in the experiment*:** Environmental factors such as temperature, dissolved oxygen (DO), pH, $NH_3^-/NH_4^+$ and NO2- were measured using the Sera test kit. The results showed that these environmental factors were always within the allowable range and suitable for the growth of fingerling eel. Temperature ranged from 25,9 ℃ - 30,6 ℃; DO content ranged from 4,8 – 6,2; pH ranged from 7,58 – 8,03; $NH_3^-$/NH4$^+$ during the eel farming process ranged from 0,015 – 0,077 mg/L, within the allowable range of < 1 mg/L; and $NO_2^-$ during the eel farming process was 0,001 – 0,013 mg/L, within the allowable range of < 0,5 mg/L. All environmental factors were suitable for the growth of fingerling eels (Boyd and Pillai, 1985).

***Effect of EPS-LP on the RPS protection value*:** The results of EPS-LP efficiency showed that supplementation with a content of 6 - 10 g/kg of feed achieved corresponding efficiency at 2 weeks and 4 weeks of 84.62% - 92.31% and 88.89% - 100% respectively (Table 1). This result shows that RPS>50% is very effective. Because, RPS value >50% is highly effective and can be recommended for use (Amend, 1981). At the same time, the RPS value results in Table 1 also show that 4 weeks of continuous PS-LP supplementation will increase the efficiency of EPS-LP utilization compared to 2 weeks, the RPS value reaching 100%. Therefore, the results of this experiment can be supplemented with 6 - 10 g/kg of feed and fed continuously for 4 weeks to achieve the highest efficiency.

**Table 1. Efficiency of EPS on the RPS (%).**

| Treatments | RPS (%) 2 week | RPS (%) 4 week |
|---|---|---|
| 4 g/kg | 38.46$^{b}$ | 44.44$^{b}$ |
| 6 g/kg | 61.54$^{ab}$ | 77.78$^{ab}$ |
| 8 g/kg | 84.62$^{a}$ | 88.89$^{a}$ |
| 10 g/kg | 92.31$^{a}$ | 100.00$^{a}$ |
| P-value | 0.004 | 0.017 |
| SE Mean | 7.69 | 9.62 |

*Note*: RPS (%): Relative percentage survival: EPS utilization efficiency of M. albus; Values in the same column with different letters are statistically different (p<0.05)

***Results of determining the density of red blood cells and white blood cells of fingerling eels blood after 2 and 4 weeks of experiment*:** The results of total red blood cells (RBC) and white blood cells (WBC) of *M. albus* after 2 and 4 weeks of experiment showed that there were statistical differences between treatments and compared with the control (p<0.05) (Table 2). RBC density tended to increase correspondingly with the concentration of EPS from 4 g/kg feed to 10 g/kg feed and with the experimental time, respectively 1.75 x $10^6$ cells/mm$^3$ to 2.43 x $10^6$ cells/mm$^3$ and from 2.57 x $10^6$ cells/mm$^3$ to 3.27 x $10^6$ cells/mm$^3$ at 2 and 4 weeks of experiment. Meanwhile, WBC density fluctuated between treatments. In the 2 weeks of the experiment, the highest WBC density was in the 6 g/kg treatment at 456.69 x $10^3$ cells/mm$^3$ and the lowest was in the 8 g/kg treatment at 149.86 x $10^3$ cells/mm$^3$. However, the WBC density after 4 weeks of the experiment showed the highest in the 8 g/kg treatment at 1,404.63 x $10^3$ cells/mm$^3$ and the lowest was in the 4 g/kg treatment at only 723.62 x $10^3$ cells/mm$^3$ (Table 2). Table 2 also shows that after 4 weeks of the experiment, the RBC and WBC densities were statistically different compared to the 2 weeks of the experiment. At the same time, the WBC density of the control increased significantly and was the highest compared to the remaining treatments. This proves that feeding of fingerling eel (*M. albus*) with EPS supplemented feed for at least 4 weeks can stimulate the immune system of fingerling eel to increase the number of WBC in the blood to inhibit and destroy the pathogen *E. tarda* T4 bacteria causing disease in *M. albus*. Overall, the results in Table 2 also show that the EPS supplemented treatment of 8 - 10 g/kg of feed gave the optimal results in this experiment.

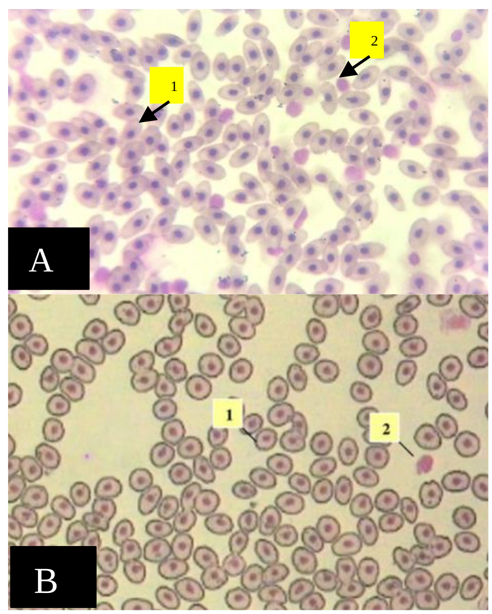


**Figure 1. A-Erythrocytes and leukocytes of Wright-Giemsa stained *M. albus*. B - Erythrocytes and leukocytes of (Hii *et al.*, 2007) [1]: erythrocytes; [2]: leukocytes.**

***The results of the different types of white blood cells on M. albus after 2 weeks and 4 weeks of experimentation*:** The results of determining the total white blood cell count and the types of white blood cells, including lymphocytes and monocytes, at 2 weeks and 4 weeks of the experiment show that there is a statistically significant difference between the

**Table 2. RBC and WBC cell density of *M. albus* determined after 2 and 4 weeks of experiment.**

| Treatments | 2 Week | | 4 Week | |
|---|---|---|---|---|
| | RBC ($10^6$ cells/mm$^3$) | WBC ($10^3$ cells/mm$^3$) | RBC ($10^6$ cells/mm$^3$) | WBC ($10^3$ cells/mm$^3$) |
| NC | $1.66^c$ | $141.39^d$ | $2.23^d$ | $1,244.74^b$ |
| PC | $1.62^c$ | $172.43^{cd}$ | $1.97^d$ | $2,566.18^a$ |
| 4 g/kg | $1.75^c$ | $374.41^b$ | $2.57^c$ | $723.62^c$ |
| 6 g/kg | $1.87^c$ | $456.69^a$ | $2.84^{bc}$ | $780.92^c$ |
| 8 g/kg | $2.15^b$ | $149.86^d$ | $3.03^{ab}$ | $1,404.63^b$ |
| 10 g/kg | $2.43^a$ | $216.27^c$ | $3.27^a$ | $908.15^c$ |
| P-value | 0.000 | 0.000 | 0.000 | 0.000 |
| SE Mean | 0.053 | 11.102 | 0.064 | 41.236 |

*Note*: NC: Negative control; PC: Positive control RBC: red blood cell; WBC: white blood cell density; different letters following the mean values in the same column are statistically different (p<0,05).

treatments ($p<0.05$). However, the count of each type of white blood cell varies greatly between treatments and increases over the duration of the experiment (Figure 2).

The results from Figure 2 indicate that the white blood cell count increased dramatically from 2 weeks to 4 weeks of the experiment. Specifically, the total white blood cell count in the treatment supplemented with EPS at a dose of 8g/kg increased from 149.86 x $10^3$ cells/mm$^3$ to 1,404.63 x $10^3$ cells/mm$^3$; the lymphocyte count increased from 71.61 cells/mm$^3$ to 2,464.35 x $10^3$ cells/mm$^3$, and the monocyte count increased from 8.01 cells/mm$^3$ to 185.84 cells/mm$^3$. At the same time, the supplementation of 8 g/kg of feed showed the highest number of lymphocytes and monocytes, which was significantly different from the control and other treatments. Therefore, to effectively stimulate the immune system of the eel fry, prebiotics can be used at a supplementation dose of 8 g/kg of feed after 4 weeks of continuous feeding. At this supplementation dose, the total white blood cell count reached 1,404.63 x $10^3$ cells/mm$^3$, lymphocytes reached 2,464.35 x $10^3$ cells/mm$^3$, and monocytes reached 185.84 x $10^3$ cells/mm$^3$.

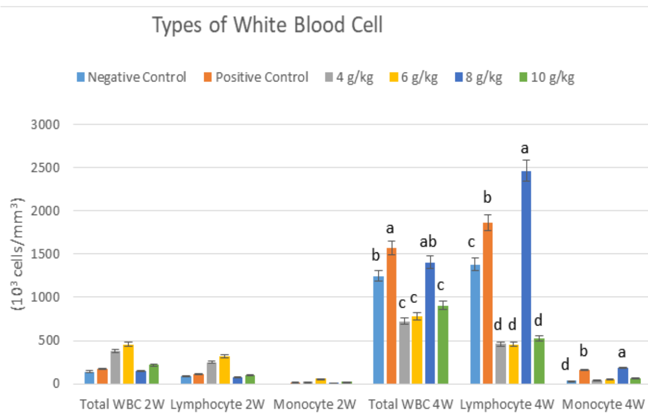


**Figure 2. The statistical results of the quantity of each type of white blood cell.**

*Note*: Different letters following the mean values in the same column are statistically different (p<0.05); 2W: 2 experimental week and 4W: 4 experimental week; WBC: white blood cell.

## DISCUSSION

Many research results have also demonstrated that the addition of immune-stimulating products - prebiotics helps increase the survival rate of aquatic species. Indeed, research results on the supplementation of alpha-lipoic acid (ALA) at 1,5 – 2 g/kg of feed in the diet of catfish susceptible to *E. tarda* bacterial infection showed a significant reduction in mortality rates compared to the control group, which were 3,% and 86,7%, respectively (Adeshina *et al.*, 2024).

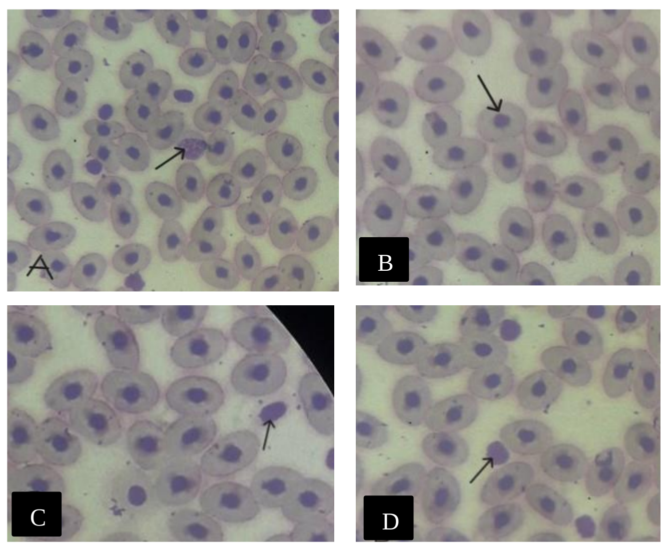


**Figure 3. A: Monocytes; B: Red blood cells; C: Platelets; D: lymphocytes (black narrow).**

In addition, the results showed that the supplementation of *L. plantarum* bacteria at a density of $10^{12}$ cells per kg of feed significantly helped the fish recover their health after being infected with *E. tarda*, achieving a survival rate of 56.7%.

The survival rate of the *M. albus* in the present study is equivalent to the results of another EPS-related experiment (Thanh *et al.*, 2021), where an 8 g/kg supplement was added to the diet of *Litopenaeus vannamei*, achieving a survival rate

of 97.3%, after 40 days of rearing, and its significantly higher than the control group with only 65.4% (Thanh *et al.*, 2021). Compared to the rest of the prebiotic experiments carried out on other aquatic fish, eel fingerling survival is the same and even higher. For carp experiment in which diet was supplemented with 0.2% MOS, the survival was up to 98.08% compared to the control group of 96.1%. Similarly, adding 0.6% MOS to the diet of carp reduced mortality rate to 16.7% from 50%.

To evaluate the potential of EPS utilization in M. albus, the RPS value is one of the key and critical factors that must be considered. The results obtained from this study indicate that the RPS value reaches a high level when EPS is added at a dose of 6 to 10 grams per kilogram of feed, and it increases further throughout the supplementation period. Specifically, RPS ranges from 61.54% to 92.31% during 2 consecutive weeks of EPS supplementation, and this value increases to 77.78% to 100% when supplementation continues for 4 consecutive weeks.

Based on these findings, it can be concluded that EPS can be effectively used for disease prevention in M. albus at a dose of 6 to 10 grams per kilogram of feed and supplemented continuously for a duration of 4 weeks. In comparison with other studies on RPS values, this research demonstrates that a higher protective coefficient is achieved with a lower concentration of supplementation.

For example, in one study, concentrated extracts of cinnamon bark (Cinnamomum verum) were added to the feed of tilapia (Oreochromis spp.) at a concentration of 20 grams per kilogram, achieving an RPS of only 51.4% (Quyen *et al.*, 2023). Similarly, another experiment involved adding blood vine (Spatholobus suberectus) and indigo plant (Isatis indigotica) to the feed to enhance resistance against the bacterium S. agalactiae, which causes disease in tilapia, and achieved RPS values of 75% and 62.5%, respectively.

Evaluation of hematological parameters plays a crucial role in analyzing the health status of the rice eel (Monopterus albus). According to (Quyen *et al.*, 2023), the number of red blood cells (RBCs) in the blood of freshwater fish varies significantly depending on factors such as developmental stage, physiological condition, and activity levels of the fish, ranging from 1 to 3.5 million cells per cubic millimeter. In contrast, the number of white blood cells (WBCs) is generally 10 to 100 times smaller than the number of RBCs. Compared to the RBC in similar fish species, which has been reported as $3.7 \times 10^6$ cells per microliter (Hue *et al.*, 2017), this study found that after four weeks of feeding rice eel with prebiotics, the RBC density at doses of 8 and 10 grams per kilogram of feed reached 3.03 × 10^6 and $3.27 \times 10^6$ cells per cubic millimeter, respectively. Additionally, supplementary feeding for two consecutive weeks at the same doses showed that RBC decreased to $2.15 \times 10^6$ and $2.43 \times 10^6$ cells per cubic millimeter, respectively.

The findings of this study revealed that the RBC in fingerling eels was similar to the RBC in other bony fish species such as catfish and anchovy (Dung, 2010; Hang and Hoa, 2020; Hue *et al.*, 2017). According to, the RBC in bony fish was determined to be $2.289 \times 10^6$ cells per cubic millimeter. For healthy Pangasius fish, the RBC and WBC counts were reported as $2.27 \times 10^6$ and $1.0 \times 10^6$ cells per cubic millimeter, respectively (Dung, 2010). Moreover, for Pangasius fish fed with 1% inulin, the RBC density was reported as $1.99 \times 10^6$ cells per cubic millimeter (Hang and Phuong, 2020). The RBC findings in this study were also consistent with a study in which 3% pomegranate extract was added to Pangasius feed, resulting in an RBC density of $2.99 \times 10^6$ cells per cubic millimeter (Hang and Hoa, 2020).

On the other hand, the RBC and WBC in Pangasius fish suffering from severe white liver and gill disease were reduced to $0.1 \times 10^6$ and $0.01 \times 10^3$ cells per cubic millimeter, respectively (Hrubec *et al.*, 2000). In a study on fingerling catfish (Clariidae), the RBC and WBC counts were reported as $3.10 \times 10^6$ and $25 \times 10^3$ cells per cubic millimeter, respectively, when supplemented with the probiotic Lactobacillus acidophilus at a concentration of $3 \times 10^7$ CFU/g for 12 weeks in the fish diet. In contrast, the results of Hii *et al.* (2007) on fingerling rice eels (Monopterus albus) showed that the RBC density was only in the range of 1.05 to $1.10 \times 10^6$ cells per cubic millimeter. These findings demonstrated that the RBC in this study was nearly twice the values reported by Hii *et al.* (2007) but lower than the RBC reported by Hu *et al.* (2020), which reached $7.6 \times 10^{12}$ cells per cubic millimeter when vitamin C was added to the diet of fingerling rice eels.

The results of leukocyte density determination in this study were lower than the results of leukocyte density determination of fingerling eels when supplemented with vitamin at the highest dose of 68,60 mg/kg 16,68 x $10^{10}$ cells/mm$^3$ (Hu *et al.*, 2020). But the WBC density in this study was higher than the study with 1% inulin supplementation, which only achieved 232,45 x$10^3$ cells/mm$^3$ (Zhou *et al.*, 2020). At the same time, the results of determining leukocyte density of this study were also higher than the results of the study adding 3% pomegranate extract to pangasius feed (Hang and Hoa, 2020). The types of white blood cells such as lymphocytes, platelets, and neutrophils in healthy fish and catfish suffering from white liver disease and white gills are 81 x $10^3$ cells/mm$^3$; 16,1 x $10^3$ cells/mm$^3$; 2,59 x $10^3$ cells/mm$^3$ and 2,59 x $10^3$ cells/mm$^3$; 8,07 x $10^3$ cells/mm$^3$; 0,25 x $10^3$ cells/mm$^3$; 1,24 x $10^3$ cells/mm$^3$ and 1,24 x $10^3$ cells/mm$^3$, respectively (Dung, 2010). For tilapia, the corresponding density of red blood cells and white blood cells is 1,91 – 2,83 x $10^6$ cells/μL and 21 – 154 x $10^3$ cells/μL (Hrubec *et al.*, 2000). Types of white blood cells such as monocytes, neutrophils, and lymphocytes significantly increased after 8 weeks of inulin supplementation 1% (Zhou *et al.*, 2020). The effectiveness of EPS (RPS), and the outcomes related to blood cells such as

red blood cells, white blood cells, and different types of leukocytes in this experiment indicate that supplementing with EPS at a dose of 8 g/kg of feed is the most suitable for the survival and increased resistance of *M. albus*.

***Conclusion*****:** The appropriate supplemental EPS content is 8 g/kg of feed for 4 consecutive weeks. At this supplemental level, the rice eel achieved the efficiency of EPS utilization (RPS) reached 88,89%. The density of red blood cells and white blood cells reaches the highest levels, respectively 3,03 x $10^6$ cells/mm$^3$ and white blood cells 1.404,63 x $10^3$ cells/mm$^3$; lymphocyte 2.464,35 x $10^3$ cells/mm$^3$ and monocytes 185,84 x $10^3$ cells/mm$^3$. Supplementing EPS at a dose of 8 g/kg of feed helps *M. albus* improve disease prevention efficiency through the RPS value. At the same time, it significantly increases white blood cells, especially lymphocytes, helping to protect eels against *E. tarda* T4 strain.

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***Informed consent*****:** N/A

***SDGs addressed*****:** Zero Hunger, Good Health and Well-being, Responsible Consumption and Production

***Policy referred*****:** Vietnam's Strategy for Sustainable Aquaculture Development to 2030, Vision to 2045, National Action Plan on Antimicrobial Resistance (AMR) in Livestock and Aquaculture

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